EnzyChromTM Glutamine Assay Kit (EGLN-100)
Quantitative Colorimetric Determination of Glutamine at 565 nm

DESCRIPTION

Glutamine is an amino acid synthesized in the muscle that plays major roles in protein synthesis, acid-base balance, anabolic processes and is utilized for cellular energy and as a carbon source. It is used in treatment of injury, trauma, burns, and also as a supplement for muscle growth and post-surgery healing. Simple, direct and automation-ready procedures for measuring glutamine concentration are very desirable. BioAssay Systems' EnzyChromTM glutamine assay kit is based on hydrolysis of glutamine to glutamate and colorimetric determination of the product. The intensity of the product color, measured at 565 nm, is proportional to the glutamine concentration in the sample.

APPLICATIONS

Direct Assays: glutamine in serum, plasma, urine, tissue extracts and cell culture samples.
Drug Discovery/Pharmacology: effects of drugs on glutamine metabolism.

KEY FEATURES

Sensitive and accurate. Use 20 µL sample. Linear detection range 0.023 - 2 mM glutamine in 96-well plate assay.
Convenient. The procedure involves adding a single working reagent, incubation for 40 min at room temperature, adding a stop reagent and reading the optical density. No 37°C heater is needed.
High-throughput. Can be readily automated as a high-throughput 96- well plate assay for thousands of samples per day.

KIT CONTENTS (100 tests in 96-well plates)

Assay Buffer: 15 mL NAD Solution: 1 mL
Enzyme A: 120 µL MTT Solution: 2 x 1.5 mL
Enzyme B: 220 µL Stop Reagent: 25 mL
Standard: 400 µL 100 mM Glutamine
Storage conditions. Store all reagents at -20°C. Shelf life of 3 months after receipt.
Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURE

Note: (1). this assay is based on an enzyme-catalyzed kinetic reaction. Addition of Working Reagent should be quick and mixing should be brief but thorough. Use of multi-channel

pipettor is recommended. (2). the following substances interfere and should be avoided in sample preparation: ascorbic acid, SDS (>0.2%), sodium azide, NP-40 (>1%) and Tween-20 (>1%).

1. *Standard Curve*. Prepare 2.0 mM glutamine Premix by mixing 5 µL 100 mM Standard and 245 µL distilled water. Dilute standard as follows. Transfer 20 µL standards into wells of a clear flat-bottom 96-well plate.

No	Premix + H_2O	Vol (µL)	Glutamine (mM)
1	100 µL + 0 µL	100	2.0
2	60 µL + 40 µL	100	1.2
3	30 µL + 70 µL	100	0.6
4	0 µL + 100 µL	100	0.0

Samples: add 20 µL sample per well in separate wells.
IMPORTANT: *if a sample is known to contain glutamate, a sample blank control is required. In this case, transfer an additional 20 µL sample into a separate well.*

2. *Reaction*. Spin the enzyme and reagent tubes briefly before pipetting. Fresh reconstitution is recommended. For each standard and sample well, prepare Working Reagent by mixing 65 µL Assay Buffer, 1 µL Enzyme A, 1 µL Enzyme B, 2.5 µL NAD and 14 µL MTT.
Where a sample blank is required, prepare a Blank Working Reagent by mixing 65 µL Assay Buffer, 1 µL Enzyme B, 2.5 µL NAD and 14 µL MTT (i.e. No Enzyme A). Add 80 µL Working Reagent per well to standards and sample wells. *Where appropriate, add 80 µL Blank Working Reagent to the Sample Blank wells*. Tap plate to mix briefly and thoroughly.

3. Incubate 40 min at room temperature. Add 100 µL Stop Reagent to each well. Read OD at 565 nm (520-600 nm).

CALCULATION

Subtract water (#4) blank OD from OD values for the standards. Plot DOD against standard concentrations. Determine the slope and calculate sample glutamine concentration,

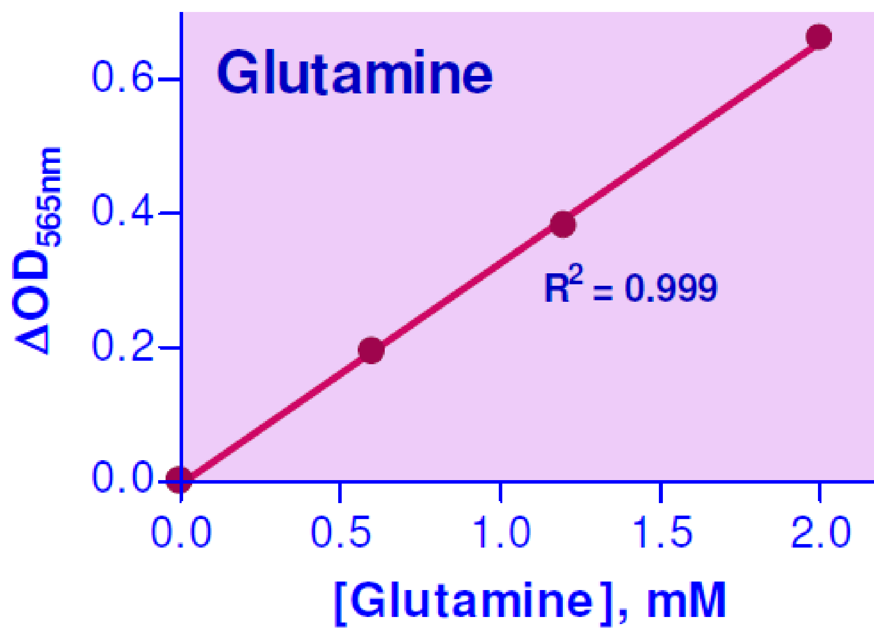
$$[\text{Glutamine}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Slope } (mM^{-1})} \times n \quad (mM)$$

where ODSAMPLE and ODBLANK are the OD values of the sample and water (if sample does not contain glutamate) or sample blank (if sample contains glutamate).

Note: if the calculated glutamine concentration is higher than 2 mM, dilute sample in distilled water and repeat the assay. Multiply the results by the dilution factor *n*. Conversions: 1 mM glutamine = 14.6 mg/dL or 146 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting (multi-channel) devices. Clear flat-bottom 96-well plates (e.g. Corning Costar) and plate reader.



Standard Curve in 96-well plate assay

LITERATURE

1. Cattaneo, MV and Luong, JH. (1993). Monitoring glutamine in animal cell cultures using a chemiluminescence fiber optic biosensor. Biotechnol Bioeng. 41(6):659-665.
2. Messer, M. (1955). A simple method for the estimation of glutamine in brain extracts. Biochim Biophys Acta. 17(1):151-152.
3. Foss, OP. (1952). A new growth medium for the cultivation and production of Clostridium welchii SR 12 in Krebs' method for the quantitative determination of glutamine and glutamic acid. Scand J Clin Lab Invest. 4(4):371-372.

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